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Effects of Low-Molecular-Weight-Chitosan on the Adenine-Induced Chronic Renal Failure Rats *in vitro* and *in vivo*

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Abstract The effects of low-molecular-weight-chitosan (LMWC) on chronic renal failure (CRF) rats induced by adenine were investigated *in vivo* and *in vitro*. Chitosan were hydrolyzed using chitosanase at pH 6–7 and 37℃ for 24 h to obtain LMWC. *In vitro*, the effect of LMWC on the proliferation of renal tubular epithelial cells (RTEC) showed that it had no cytotoxic effect and could promote cell growth. For the *in vivo* experiment, chronic renal failure rats induced by adenine were randomly divided into control group, Niaoduqing group, and high-, medium- and low-dose LMWC groups. For each group, we detected serum creatinine (SCR), blood urea nitrogen (BUN), and total superoxide dismutase (T-SOD), glutathione oxidase (GSH-Px) activities of renal tissue, and obtained the ratio of kidney weight/body weight, pathological changes of kidney. The levels of serum SCR, BUN were higher in the adenine-induced rats than those in the control group, indicating that the rat chronic renal failure model worked successfully. The results after treatment showed that LMWC could reduce the SCR and BUN levels and enhance the activities/levels of T-SOD and GSH-PX in kidney compared to control group. Histopathological examination revealed that adenine-induced renal alterations were restored by LMWC at three tested dosages, especially at the low dosage of 100 mg kg^{-1} d^{-1}.

Key words LMWC; RTECs; CRF; adenine; SCR; BUN; T-SOD; GSH-PX; renal histopathology

1 Introduction

Chitosan is a linear polymer with β(1-4) linked D-glucosamine units derived from the N-deacetylation of chitin. It has been extensively studied for delivery of anti-cancer agents, therapeutic proteins, genes, antigens, and so on due to its biological properties, such as relative non-toxicity, biodegradability, biocompatibility, cationic properties and bioadhesive characteristics (Kumari *et al*., 2010; Amidi *et al*., 2010; Sinha *et al*., 2004). However chitosan with high molecular weight shows high viscosity and low water solubility at neutral pH, which limits its use (Harish Prashanth and Tharanathan, 2007; Kittur *et al*., 2003). Its biological application depends upon its molecular weight and deacetylation degree (McGahren *et al*., 1984). Fortunately, low-molecular-weight-chitosan (LM WC), produced through chemical or enzymatic hydrolysis of chitosan has overcome these limitations, and it is easier to be absorbed by organism. Moreover, it has been identified that LMWC has many outstanding health benefits such as anti-inflammatory, immunity regulation, anti-carcinogenic, liver protection, hypolipidemic, anti-diabetic, antioxidant and anti-obesity (Chung *et al*., 2012; Maeda and Kimura, 2004; Baek *et al*., 2007; Yin *et al*., 2009).

Chronic renal failure (CRF) occurring in a variety of chronic kidney diseases (CKDs) is a common clinical syndrome, and the renal function diminishing can develop to uremia slowly. The irreversible kidney damage caused by chronic renal failure can impose an increased burden on surviving nephrons and lead to the end-stage of renal failure. Then renal dialysis or transplantation will be required (Howie, 2008). Adenine-induced CRF model provides useful information on the pathomechanism of various complications associated with a persistent uremic state (Wang *et al*., 2011). Long-term feeding of high concentration of adenine to rats can lead to toxin accumulation and metabolism disorder, resembling CRF complication in humans, and ultimately leads to renal failure (Yokozawa *et al*., 1977; Yokozawa *et al*., 1986).

Tubular interstitial fibrosis is a common pathological mechanism of various CKD developing to renal failure (Arata *et al*., 2005). Normal functional tubular epithelial cells in kidney fibrosis are significantly reduced or totally disappear, which can be caused by damage factors such as uremic toxin serum, or trans-differentiation (Guo *et al*., 1999). Primary renal tubular epithelial cells (RTECs) have been cultured successfully using sieve conjugating enzyme digestion (Wang *et al*., 1999; Cartier *et al*., 1993). They can be applied in studying the factors and mechanisms responsible for diseases and disorders of the kidney.

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Chitosan supplements might be an effective treatment for renal failure, while LMWC can help repair the aristolochic acid-induced renal lesions in mice (Jing *et al*., 1997; Chang *et al*., 2011). However, the effects and mechanism of LMWC in renal damage induced by adenine is not clear. This study used LMWC prepared by hydrolysis of chitosan using chitosanase and investigated the effects of LMWC on the factors influencing renal function and renal histopathology for adenine-induced renal lesions in vivo and in vitro.

2 Materials and Methods

2.1 Materials

Lower-molecular-weight-chitosan (LMWC) (M.W. < 1.3 kDa, deacetylation degree >46.58%) was produced in our laboratory. Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (Ham) (1:1) D-MEM/F-12 (powder) was purchased from Invitrogen Corporation. Defined fetal bovine serum (FBS) was purchased from BeiJing Yuanhengjinma Biotechnplogy kaifa Co., Ltd. MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was from Sigma-Aldrich; Trypsin powder was from Gibco Corporation; 96-microtiter plates were from COSTAR Corporation in USA; metal sieves of 80 mesh (0.180 mm diameter) and 120 mesh (0.125 mm diameter) were purchased from Huangsheng Biotechnology Co., Ltd.

Adenine (Amresco 0183-100G) was from Amresco. Carboxymethylcellulose (CMC) was fromTexas Sea Fiber Co., Ltd. SCR, SUN, GSH-PX and T-SOD kits were purchased from NanJing Jiancheng Biotechnology Co., Ltd. Wistar rats (male and female, 200 g ± 20 g) were purchased from Qingdao Institute for Drug Control, China.

2.2 The Effect of LMWC on Primary RTECs

2.2.1 Isolation and culture of rat proximal tubular segments

To isolate the proximal tubular segment, kidneys were obtained from rats with 12-hour-hunger and free water sterility and placed in D-Hank's buffered salt solution with antibiotics (Gentamycin sulfate). Briefly, kidney cortex was separated and washed with D-Hank's to remove blood and urine, and was then chopped into small pieces and pressed through metal sieves of 80 mesh, grinded and washed thoroughly with Hank's buffered salt solution, to remove glomeruli and larger fragments. The filtrate was then flitered through metal sieves of 120 mesh to collect blocks on mesh screen in centrifuge tube containing D-Hank's, and centrifuged at 800 r min^{-1} for 5 min.

After centrifugation, the supernatant was discarded and the precipitate was resuspended in 0.1% collagenase I and was digested for 20 min at 37℃. Then the same volume of DMEM/F12 containing 10% FBS was added to terminate digestion, followed by centrifuging at 1000 r min^{-1} for 5 min, and the supernatant was removed carefully. Rat proximal tubular segments were produced in 6-well tissue culture plates using pure serum in a humidified 5% CO_2/95% air at 37℃. After 4 h, DMEM/F12 was added to each well and the medium was changed after 72 h. Then the medium was changed every 48 h until the proximal tubular epithelial cells reached confluence.

2.2.2 The effects of LMWC on RTECs

Emigration cells from renal tubular segments were cultured to confluence, and then were detached with 0.25% Trypsin-0.02% EDTA and the cell masses were blew completely. Cell number was counted with haemocytometer. Then the cells were cultured in triplicate at a density of 1×10^4 cells per mL in 96-well plates with 200 μL $well^{-1}$ for 24 h at 37℃ in 5% CO_2 incubator. Then the cells were cultured in D-MEM/F-12 with five different concentrations of LMWC (10, 100, 250, 500, 1000 μg mL^{-1}). In the control group, there was no LMWC in the medium. After the cells were cultured for 2, 4, 6 d, 20 μL of MTT reagent (5 mg mL^{-1} D-Hank's) was added and incubated at 37℃ for 4 h. Then the supernatant was removed and 100 μL $well^{-1}$ of DMSO was added. Then, the plates were shaken at room temperature for 10 min and the absorbance was measured at 570 nm. Relative metabolic activity compared to control cells was analyzed.

2.3 The Effect of LMWC on the Rats

2.3.1 Animals

Wistar male and female rats, approximately 180–220 g, were purchased from the Experimental Animal Center of Shandong Lukang Pharmaceutical Co., Ltd., the experimental animal production license number being SCXK Lu 20080002. The animals were housed under standard laboratory conditions (12 h light and 12 h dark) with controlled temperature (24℃ ± 3℃), and allowed to have free access to a standard food and water supply during the experiment period.

2.3.2 The preparation of adenine suspension and induction of renal failure

Adding distilled water to configure 0.5% *CMC* solution, and then adenine was added to 0.5% CMC solution to configure 2.5% suspension.

All rats (n=56) were randomly divided into 6 groups after 1 week of adaptation with the same male and female rats in each group. In normal control group (Group I) there were 12 animals; in CRF model group (Group II) there were 20 animals; in Naoduqing group (Group III) there were 6 animals; in LMWC High (Group IV)/Medium (Group V)/Low (Group VI) groups there were 6 animals respectively. The control rats were treated with 2 mL of 0.5% CMC solution. The rats, in other groups were gavaged with adenine suspension at a dose of 250 mg kg^{-1} d^{-1}, once a day for the first two weeks and once every other day for the following two weeks. At the end of modeling, abdominal aortic blood, *SUN* and *SCR* in the blood serum were tested. The significant difference between the model group and control group confirmed that the CRF model was ready.

2.3.3 Animal treatment

After the CRF model was ready, normal control and CRF model group rats were treated with 2 mL distilled water; the Naoduqing group (positive control group) were treated with 2 mL Naoduqing at doses of $2\,g\,kg^{-1}\,d^{-1}$; the LMWC High, Medium and Low groups were gavaged with 2 mL LMWC at doses of 300, 200, and $100\,mg\,kg^{-1}\,d^{-1}$.

After the rats were cultured for 4 weeks, they were anaesthetized using pentobarbital ($50\,mg\,kg^{-1}$ BW) and the blood samples were collected by abdominal aortic with lancets. Blood samples were centrifuged at 4℃ with 2000 $r\,min^{-1}$ for 15 min. Then the serum was stored at −20℃ for further assay. After the blood was taken, the rats were killed and the kidneys were isolated. One gram of kidneys were homogenized, in 0.86% NaCl solution (1:9). Homogenates were centrifuged at $3500\,r\,min^{-1}$ for 20 min at 4℃ to remove cell debris and then the supernatant was collected and stored at −20℃ to determine GSH-PX, T-SOD enzymatic activities. A portion of kidney tissue was fixed in 10% formalin and embedded in paraffin wax for histological examination. Sections were cut at 5 μm thickness, stained with hematoxylin and eosin (H&E), and examined under light microscopy with the magnification of 100.

2.4 Statistical Analysis

All data are expressed as mean ± S.D. and analyzed using Student's t-test on SPSS 13.0 software. Differences were considered to be statistically significant at $P<0.05$, and to be highly statistically significant at $P<0.01$.

3 Results

3.1 Morphological Observation of Primary RTECs

The morphology of tubular segments under inverted microscope is shown in Fig.1A. The cultured tubular segments were mostly (90%) adhered after 3 days to a few of oval renal tubular epithelial cells surrounding them (Fig.1B). The number of cells increased gradually on the 5th day (Fig.1C) and 7th days (Fig.1D), and spread on the culture plate with a total confluence on 9–10th days (Fig.1E). Cells were large size, multilateral pebbles sample, transparency and refraction sex strong.

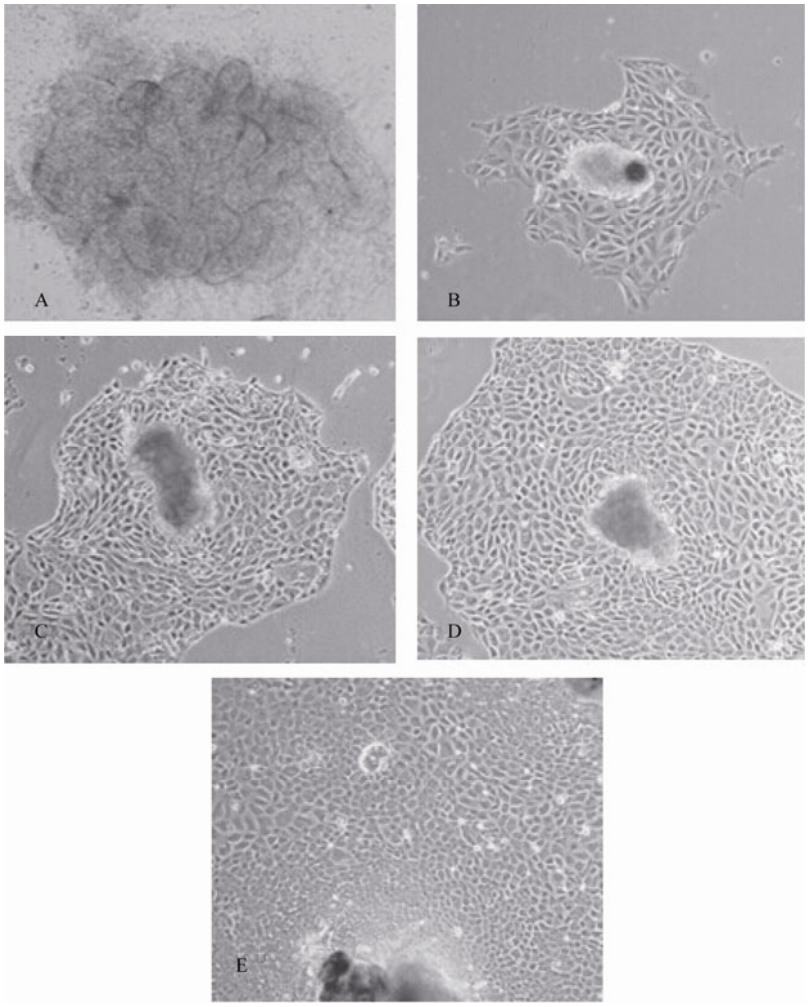

Fig.1 Morphology of primary renal tubular epithelial cells under microscope. (A) Renal tubular segment on the surface on day 3; (B) The morphology of renal tubular epithelial cells migrated out of the segment after being cultured for 3 days; (C) The spread of cells on day 5; (D) More cells attached to the surface on day 7; (E) The cells were nearly 100% confluent on day 9.

3.2 Effect of LMWC on the Proliferation of RTECs *in vitro*

Cells were incubated in media with six different concentrations of LMWC (0, 50, 125, 250, 500, 1000 μg mL^{-1}). After the cells were cultured for 24 h, MTT-test results (Fig.2) showed that within the tested concentration, no significant difference of absorption value was observed compared with control group, and the cells began to enter into the logarithmic phase.

After the cells were cultured for 72 h, from the MTT-test results (Fig.2), the cell proliferation rates were 97.84%, 105.04%, 113.91%, 105.24%, 86.75% respectively when they were cultured with different concentrations of LMWC. Compared with the control cells, the difference of the absorption value was significant when the cells were cultured with 250 μg mL^{-1}, 1000 μg mL^{-1} LMWC. Morphology of cells exposed to different concentrations of LMWC (50, 125, 250, 500, 1000 μg mL^{-1}) at the 72nd hour is shown in Fig.3. The numbers of cells with different concentrations of LMWC were different, and the largest number of cells was observed when they were cultured with 250 μg mL^{-1} LMWC (Fig.3D).

After the cells were cultured for 120 h, MTT-test results (Fig.2) indicated that that OD values of cells cultured with 250, 500 μg mL^{-1} of LMWC were significantly higher than those for the control group. The cell proliferation rates were 102.19% 107.62%, 119.54%, 114.73%, 96.88%, respectively. Therefore, LMWC with 125–500 μg mL^{-1} could promote the proliferation of RTEC differently, while the effect was most significant at 250 μg mL^{-1}. The RGR of each LMWC-treated group was 0 grade or 1 grade according to the evaluation standard of cytotoxicity (Table 1), indicating that LMWC is a non-cytotoxic biological material and in line with the application standard.

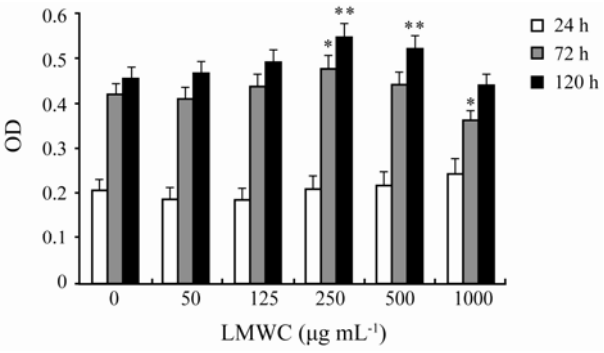

Fig.2 *In vitro* growth of LMWC on the RTECs measured by MTT-test. Cells were exposed to different concentrations (50, 125, 250, 500, 1000 μg mL^{-1}) of LMWC for 24, 72, 120 h. The results are mean ± SD of 2 separate experiments (n = 6). Asterisks indicate statistically (extremely) significant differences (**P < 0.01, *P < 0.05) compared with cells in control group.

Table 1 The evaluation standard of cytotoxicity

Toxicity ratings	RGR (%)
0 grade	≥100
1 grade	80–99
2 grade	50–79
3 grade	30–49
4 grade	0–29

Notes: Evaluation standard: Relative cell growth rate (RGR) = absorbance value of LMWC group/absorbance value of control×100%. Grades 0 and 1, qualified; Grade 2, combined with morphological analysis and comprehensive evaluation; Grades 3 and 4, unqualified.

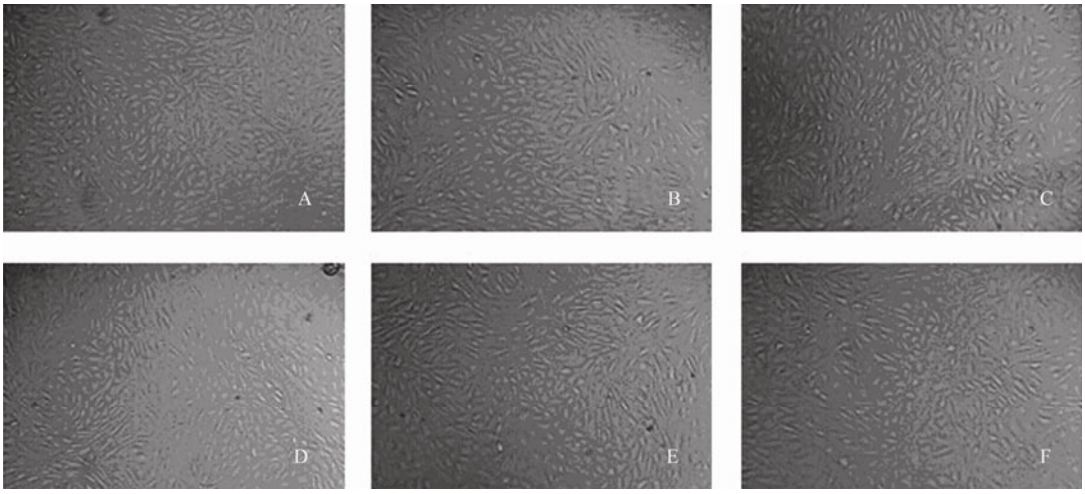

Fig.3 Effect of LMWC on the morphology of RTECs treated for 72 h. Microscope observation of cells exposed to different concentrations of LMWC (A) 0, (B) 50, (C) 125, (D) 250, (E) 500, (F) 1000 μg mL^{-1} for 72 h. The number of cells cultured with different concentrations of LMWC was different.

3.3 Changes of Body Weight and the Ratio of Kidney Weight/Body Weight

The changes in body weight and the ratio of kidney weight/body weight (g g^{-1}) were examined in normal control group, model group and LMWC groups animals during experimental period. The body weight of animal groups subject to adenine decreased continually with an average loss of 5.98% body weight by the end of modeling, while the normal control group gained 10.86% body

weight (Table 2). The ratio of kidney weight/body weight was 0.0322±0.0019, significantly higher ($P<0.01$) in the model group compared to that in the normal control groups with the ratio 0.0054±0.0003 at the end of modeling. After treatment, the animals in each group gained 17.41%, 25.81%, 27.2%, 15.45%, 21.33%, 33.14% body weight (Table 2), respectively, but the ratio of kidney weight/ body weight showed minimal change, compared to the model group in all LMWC treatment groups (Fig.4).

Table 2 Change of body weight during experimental period

Period	Normal control	CRF model	Positive control	LMWC High	LMWC Medium	LMWC Low
0 week	227.22±13.27	212.67±18.33	211.67±18.03	221.44±19.79	223.56±19.47	205.67±16.63
4 week	251.83±19.30	192.22±16.75**	195.63±23.96**	214.56±21.78**	204.67±22.62**	203.33±20.63**
8 week	295.67±35.92	241.83±22.00	269.25±13.87	255.67±21.96	271.20±15.56	273.83±24.09

Notes: Body weight is presented before modeling, at end of modeling and treatment (*$P<0.05$, **$P<0.01$ *vs*. normal control).

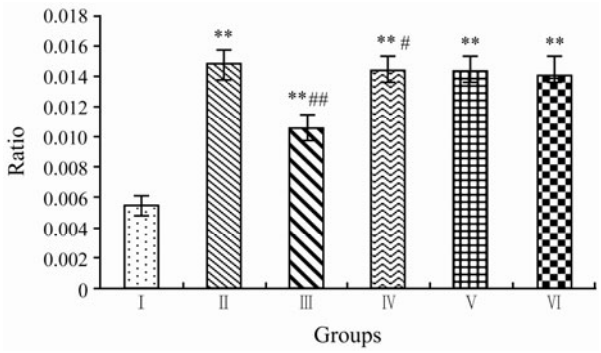

Fig.4 Effect of the LMWC on the ratio of kidney weight/body weight at week 8. The results are expressed as ratio (3–6 determinations), mean±SD, *$P<0.05$, **$P<0.01$ *vs*. normal control; $^{\#}P<0.05$, $^{\#\#}P<0.01$ *vs*. model). Normal control group, Group I; CRF model group, Group II; Naoduqing group, Group III; LMWC High, Group IV; LMWC Medium, Group V; LMWC Low, Group VI.

3.4 Effect of LMWC Ingestion on Serum Creatinine (SCR) and Urea Nitrogen (BUN) Levels

The SCR and BUN levels of adenine, induced CRF rats were 180.40 μmol L^{-1} and 21.38 mmol L^{-1} respectively, significantly higher than those of the normal rats (63.80 μmol L^{-1}, 7.16 mmol L^{-1}) at the end of modeling ($P<0.01$), indicating that the rat CRF model worked successfully. The SCR and BUN levels are important indicators of renal function.

After treatment with LMWC for 4 weeks, the model group had higher SCR ((86.97±1.98) μmol L^{-1}) and BUN ((10.84±0.55) mmol L^{-1}) levels than the control group ((67.54±8.26) μmol L^{-1} and (6.15±0.18) mmol L^{-1}) and the SCR and BUN levels of treated rats were lower than those of model group rats (Fig.5). The SCR level of High-, Medium- and Low-LMWC groups were (85.66±3.63) μmol L^{-1}, (63.62±6.02) μmol L^{-1}, and (65.03±4.61) μmol L^{-1}, respectively. The SCR concentrations of Medium- and Low-LMWC groups were significantly different ($P<0.01$) compared to the model group, and better than the positive control group (Fig.5A). The BUN levels in LMWC treatment groups were (8.74±0.96) mmol L^{-1}, (8.25±0.90) mmol L^{-1}, and (7.85±0.33) mmol L^{-1}, respectively (Fig.5B). They were all significantly lower than that of the model group ($P<0.01$).

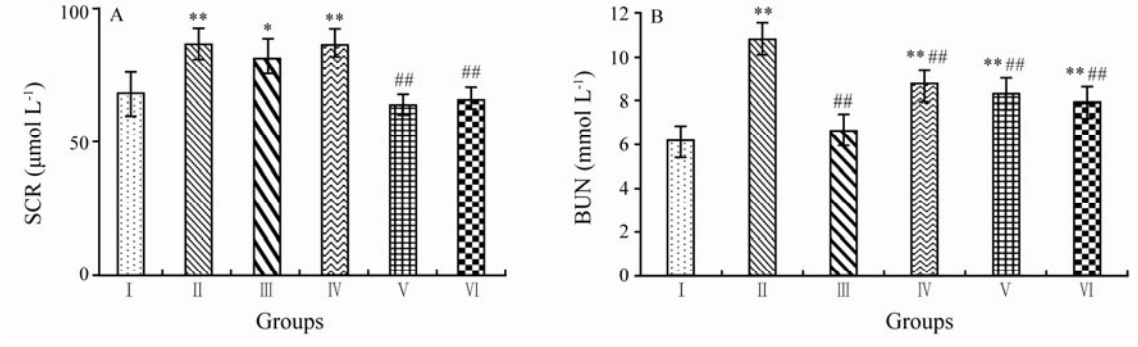

Fig.5 Effect of LMWC on Serum Creatinine (SCR) and Urea Nitrogen (BUN) levels at week 8. The results are expressed as concentration values in serum (3–6 determinations), mean±SD, *$P<0.05$, **$P<0.01$ *vs*. normal control; $^{\#}P<0.05$, $^{\#\#}P<0.01$ *vs*. model). Normal control group, Group I; CRF model group, Group II; Naoduqing group, Group III; LMWC High, Group IV; LMWC Medium, Group V; LMWC Low, Group VI.

3.5 Effect of LMWC on T-SOD and GSH-PX Activities in Rat

T-SOD and GSH-PX in kidney provide the primary enzymatic antioxidant defenses. The effect of LMWC on T-SOD in kidney was observed after treatment (Fig.6A). T-SOD activity was significantly reduced in model group (430.71±13.45 U mg^{-1} protein) compared with normal control group ((229.85±13.72) U mg^{-1} protein) ($P<0.01$). All LMWC treatment groups enhanced T-SOD activity

compared with model group. The T-SOD activities were $(276.33 \pm 35.08)\,\mathrm{U\,mg^{-1}}$ protein, $(290.39 \pm 34.97)\,\mathrm{U\,mg^{-1}}$ protein, and $(357.11 \pm 29.34)\,\mathrm{U\,mg^{-1}}$ protein, respectively. Significant differences were observed in those groups compared with normal control group ($P < 0.01$).

Moreover, the GSH-PX markedly reduced by gavage of adenine could be improved by LMWC treatment, and the activites in medium- and low-LMWC groups were $264.90 \pm 25.96\,\mathrm{U\,mg^{-1}}$ protein and $277.13 \pm 18.96\,\mathrm{U\,mg^{-1}}$ protein (Fig.6B).

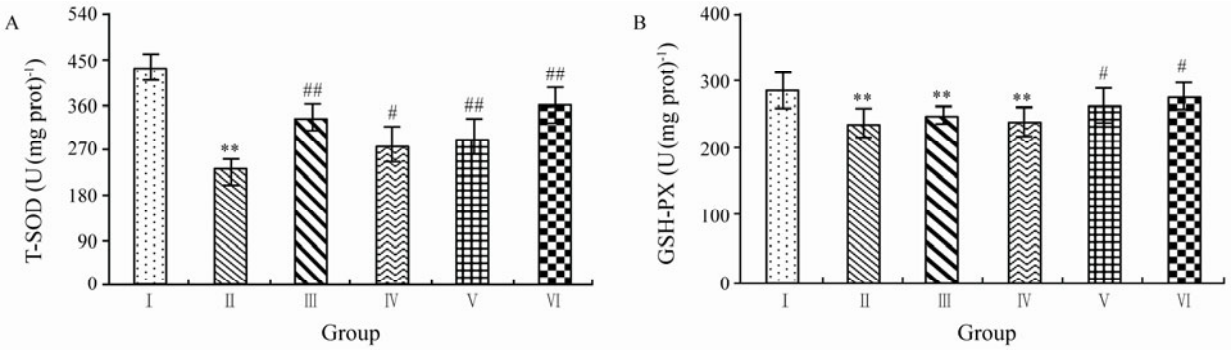

Fig.6 Effect of LMWC on enzymatic activities/levels in rat (T-SOD and GSH-PX) in 8 weeks. The results are expressed as activity values in Renal tissue (3–6 determinations, mean ± SD, $^{*}P < 0.05$, $^{**}P < 0.01$ *vs*. normal control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs*. model). Normal control group, Group I; CRF model group, Group II; Naoduqing group, Group III; LMWC High, Group IV; LMWC Medium, Group V; LMWC Low, Group VI.

3.6 Morphology of Renal Tissue

In normal control rats, all renal tissue samples were histologically normal (Fig.7A). Glomerular and tubular lesions were not found. Adenine exerted a strong nephrotoxic effect on the rat, due to the fact of renal lesions and severe levels of change revealed by microscopy (Fig.7B). Large aggregates of characteristic brown adenine metabolism crystals (2,8-dihydroxy-adenine) produced by the oxidation of xanthine oxidase distended tubules, tubular dilatation, flattening of tubular epithelial cells, some tubular atrophy, tubular degeneration associated with interstitial fibrosis bundle or meshed into pieces, and infiltration of inflammatory cells at the interstitium and perivascular tubular necrosis characterized these changes. Moreover, glomerular lesions, such as global sclerosis, cysts expanding and intimal thickening were observed.

Treatment with LMWC partially alleviated the renal lesion. However, the severity of toxic nephropathy in high dose (Fig.7D) and medium dose (Fig.7E) was higher than that in low dose (Fig.7F). There were reduced metabolic crystals, tubular moderate degeneration, moderate interstitial fibrosis and reduction of inflammatory cells in the LMWC Low group, which was relatively mild compared to the severity of lesions observed in the model group. Tubular moderate degeneration and more inflammatory cells were observed in LMWC Medium group. In High-LMWC group, tubular dilatation degeneration or atrophy and interstitial fibrosis moderately severe were observed. In all LMWC treatments, glomerular lesions were not mitigated obviously.

4 Discussion

In vitro, the effect of LMWC on the growth of renal tubular epithelial cells (RTECs) was monitored utilizing detection principle of MTT. We conclude that LMWC could promote growth of renal tubular epithelial cells within a certain range of concentrations and was non-cytotoxic. Yuan demonstrated that 50% N-Acetylated LMWC was specifically taken up by renal tubular cells, where the megalin receptor would likely mediate its binding and uptake (Yuan *et al*., 2009). LMWC in this study might be more easily absorbed by cells because of this specificity having effects on RTEC.

In vivo, LMWC had an effect of reducing the SCR and BUN levels and elevating the activities/levels of T-SOD, GSH-PX in kidney compared to model group. As serum creatinine (SCR) and Urea nitrogen(BUN) levels are important indicators of renal function, a reduction in these levels suggests that renal functionality is improved (Chang *et al*., 2011; Takashi *et al*., 2004). In our research, histopathology observation of CRF rats induced by adenine showed kidneys were diseased or damaged, and the SCR and BUN levels in model group were significantly higher than those in the normal rats. Diseased or damaged kidneys cause an elevated SCR and BUN because renal dysfunction diminishes the ability to filter creatinine and clear urea from the bloodstream (He *et al*., 2009). A number of researchers have also demonstrated that chitosan can bind urea, ammonium, and certain acidic substances but not bind creatinine *in vitro* experiments (Maezaki *et al*., 1993). It has been proved that LMWC decreases serum creatinine due to restoring the function of renal tubular cells with LMWC, which can reduce serum creatinine levels (Chang *et al*., 2011). In our research the results indicated that LMWC decreases serum creatinine and urea nitrogen; therefore, promoting proliferation of renal tubular epithelial cell to supplement necrosis and degeneration tubular might also reduce serum creatinine levels, and LMWC binding urea could decrease

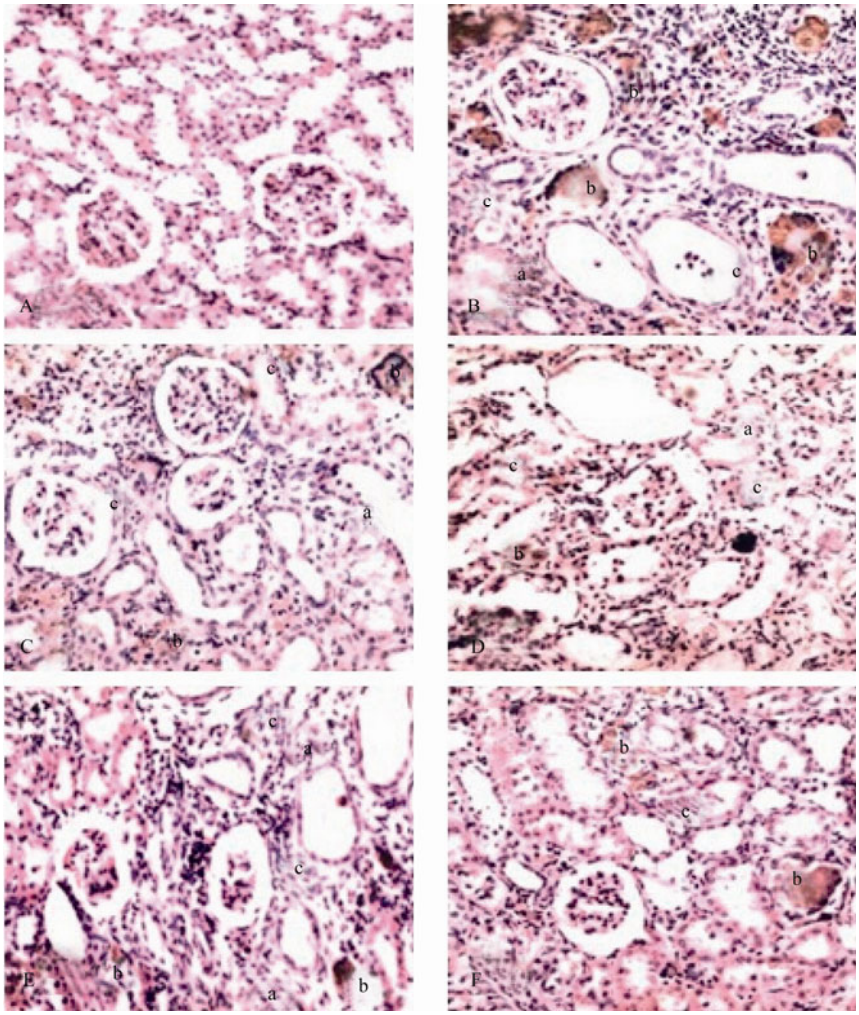

Fig.7 Sections were stained with hematoxyline-eosin (H-E, 100×). Light microscopy of renal tissue of normal rats after gavage of water(A), CRF model rats after adenine-treated without treatment (B), positive control group rats after adenine-treated with Niaoduqing treatment for 4 weeks (C), LMWC at a high (D), medium (E) and low (F) dose treatment for 4 weeks. (a): tubular atrophy or degeneration, (b): brown crystals, (c): interstitial fibrosis.

the degree of SUN.

Normal renal tubular epithelial cells metabolism can generate oxygen free radicals that can be rapidly cleared by enzymatic antioxidants in kidney. Certain research results clearly show that free radicals can interact with renal epithelia, damage the renal cells and membranes, and lead to tubules dysfunction or damage (Grases *et al*., 1998). Histopathology observation of CRF model rats shows that the renal tubular of residual nephron causes the compensatory hypertrophy injury, which can lead to accumulate more free radicals and overwhelm antioxidant defense mechanisms.

T-SOD can eliminate superoxide anion radical (O_2^-) to protect cells from oxygen free radicals damage. It was revealed that GSH-PX can break down H_2O_2 and other peroxide, which are derived from the oxidation membrane phospholipids, into water and oxygen, thus limiting the production of the highly reactive hydroxyl free radical (Amstad *et al*., 1994; CeballosPicot *et al*., 1996). Hence T-SOD and GSH-PX act in tandem and provide the primary enzymatic antioxidant defenses. In this study, the T-SOD, GSH-PX activities in model group were significantly lower than those of the normal rats, and all the LMWC groups could increase the activities of T-SOD, GSH-PX. It is reported that the low molecular weight chitosan (< 1 kDa) can inhibit NO production in murine macrophage cell line with strong inflammatory stimuli and bacterial lipopolysaccharide (Niranjan *et al*., 2006). Moreover, the low molecular weight chitosan (< 3.5 kDa) can reduce the production of pro-inflammatory cytokine such as Interleukins lymphocyte-1a (IL-1a) and Tumor Necrosis Factor-a (TNF-a) (Kim *et al*., 2006). Thus, LMWC has protective effect on proximal tubules and kidney tissue by increasing kidney cells, or decreasing tissue damage mediated by the reaction of free radicals, NO and pro-inflammatory cytokine. Moreover, the activities/levels of T-SOD, GSH-PX in renal tissue are also increased. It is concluded that LMWC has antioxidant effect on reducing oxidative stress by enhancing antioxidant enzyme activity in the renal tissue.

Based on pathological findings in this study, adenine impairs renal tissue, the glomerular and tubular lesions. Administration of LMWC for CRF rats alleviates renal lesions in rats when using a suitable dosage (100 mg kg^{-1}

d^{-1}).

5 Conclusion

Low-molecular-weight-chitosan (LMWC) can promote the proliferation of renal tubular epithelial cells without cytotoxic effect *in vitro*. It can effectively slow down adenine-induced renal tissue damage by reducing SCR and BUN levels and enhancing the activities/levels of T-SOD and GSH-PX in kidney *in vivo*. Thus LMWC can be applied to prevent or repair renal failure.

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